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# Kinetic Modeling of β-Chloroprene Metabolism: II. The Application of Physiologically Based Modeling for Cancer Dose Response Analysis

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**β-Chloroprene (2-chloro-1,3-butadiene; CD), which is used in the synthesis of polychloroprene, caused significant incidences of several tumor types in B6C3F1 mice and Fischer rats, but not in Wistar rats or Syrian hamsters. This project investigates the relevance of the bioassay lung tumor findings to human health risk by developing a physiologically based toxicokinetic (PBTK) model and exploring a tissue specific exposure-dose-response relationship. Key steps included identification of the plausible genotoxic mode of action, experimental quantification of tissue-to-air partition coefficients, scaling of *in vitro* parameters of CD metabolism for input into the PBTK model, comparing the model with *in vivo* experimental gas uptake data, selecting an appropriate tissue dosimetric, and predicting a corresponding human exposure concentration. The total daily milligram amount of CD metabolized per gram of lung was compared with the animal bioassay response data, specifically combined bronchiolar adenoma/carcinoma. The faster rate of metabolism in mouse lung agreed with the markedly greater incidence of lung tumors compared with the other rodent species. A lung tissue dose was predicted for the combined rodent lung tumor bioassay data at a 10% benchmark response. A human version of the PBTK model predicted that the lung tissue dose in humans would be equivalent to continuous lifetime daily exposure of 23 ppm CD. PBTK model sensitivity analysis indicated greater dependence of model predictions of dosimetry on physiological than biochemical parameters. The combined analysis of lung tumor response across species using the PBTK-derived internal dose provides an improved alternative to default pharmacokinetic interspecies adjustments for application to human health risk assessment.**

*Key Words:* **2-chloro-1,3-butadiene; PBTK or PBPK modeling; benchmark dose; liver; lung; mouse; rat; hamster; human.**

β-Chloroprene (2-chloro-1,3-butadiene; CD) is a chlorinated four-carbon diene monomer used in the manufacture of polychloroprene rubber. This synthetic rubber is important for automotive applications (belts, hoses, gaskets), adhesives, wire and cable insulation, structural shock absorbers and other load-bearing products, dipped goods (gloves), and other manufactured products (e.g., wetsuits). The global production of CD is estimated at 247.7 kt/year.[2] Human exposure may occur during monomer synthesis, transport and polymerization processes. The route of exposure is predominantly by inhalation owing to considerable volatility (Bp 59.4°C, vapor pressure 100 mm Hg @ 6.4°C).

Chronic inhalation studies in B6C3F1 mice and Fischer rats showing that CD is a multisite carcinogen (Melnick *et al.*, 1999), contrasted with the lack of tumors observed in Wistar rats and Syrian hamsters (Trochimowicz *et al.*, 1998). In the bioassay with Wistar rats and Syrian hamsters, inhalation exposures were to 0, 10, or 50 ppm chloroprene (6 h/day, 5 days/week for up to 104 weeks and 72 weeks, respectively; Trochimowicz *et al.*, 1998). For Wistar rats, an increased incidence of mammary gland fibroadenoma was observed at 50 ppm. No increase in tumor incidence of any tissue type was observed in hamsters. In a bioassay with male and female B63CF1 mice and Fischer/344N rats, exposures were to 0, 12.8, 32, or 80 ppm (6 h/day, 5 days/week for up to 104 weeks; NTP, 1998). Tumors in mice (male and female) were observed in the lung, circulatory system, Harderian gland, and forestomach. Tumors were also observed in the kidney (male mice only) and mammary gland, skin, mesentery, Zymbal gland, and liver (female mice only). Tissues affected in Fischer rats included the oral cavity, thyroid gland, and kidney (male and female), lung (male only) and mammary gland (female only). The lung tumor response for combined bronchiolar adenoma or carcinomas was greater in the male B6C3F1 mouse than male Fischer rat (Melnick *et al.*, 1999; Melnick and Sills, 2001).

The mechanistic steps by which CD exposure leads to rodent tumors, while not understood fully, strongly suggest a genotoxic mode of action. CD is mutagenic in bacterial reverse

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[2] Data for 2002 from International Institute of Synthetic Rubber Producers, Houston, TX.

mutation assays. Other *in vitro* and *in vivo* studies for either gene mutation or structural chromosomal damage were negative (see review by Valentine and Himmelstein, 2001). The *in vitro* microsomal metabolism of CD by cytochrome P450 oxidase produces reactive intermediate epoxides; one of these, (1-chloroethenyl)oxirane, was positive in a bacterial reverse mutation assay, but not clastogenic in an *in vitro* micronucleus screening study using cultured Chinese hamster V79 cells (Himmelstein *et al.*, 2001a,b). This metabolite recently was shown to have *in vitro* reactivity toward nucleosides and calf thymus DNA (Munter *et al.*, 2002). A second epoxide metabolite, (2-chloro-2-ethenyl)oxirane, was inferred from carbonyl compounds found as aqueous hydrolysis products of this epoxide (Cottrell *et al.*, 2001). The metabolic and genotoxic profile of CD is consistent with that of the chemical analogs 1,3-butadene and isoprene. However, the physiologically based toxicokinetic (PBTK) models developed for 1,3-butadiene or isoprene have seen limited application to tumor or nontumor dose response modeling (Melnick and Kohn, 2000; Sweeney *et al.*, 2001). The metabolism of CD is also analogous to ethylene and vinyl chloride as the two-carbon analogs of each end of the CD molecule. PBTK models have been developed for ethylene and vinyl chloride, and successfully applied to dose response modeling for vinyl chloride (Clewell *et al.*, 2002). In the current study, the application of PBTK modeling acts as an important tool for understanding CD uptake and metabolism with respect to low-to-high exposure concentrations and interspecies differences.

In the absence of definitive epidemiology studies (Acquavella and Leonard, 2001), the animal bioassay data is important for estimating the cancer risk in humans exposed to CD. The purpose of this research is two-fold: (1) to implement a PBTK model for use in estimating a relevant measure of internal dose, similar to that which has been done for other chemicals (Clewell *et al.*, 2002), and (2) to explore a biologically based approach for the CD inhalation dose-response assessment in humans. Recent experiments comparing the rates of CD metabolism in liver and lung microsomes provided the metabolic parameters needed for model development (Himmelstein *et al.*, 2004).

## MATERIALS AND METHODS

***Chemicals.*** Because CD is a rodent carcinogen, care was taken to handle it using adequate ventilation and personal protective equipment. The source of CD (>99%) was the same as described previously (Himmelstein *et al.*, 2001a, 2004). Other chemicals used were of the highest purity available.

***Animals.*** The selection of rodent species and rat strains was based on those used in the inhalation bioassay studies. Rodents were purchased from Charles River Laboratories (Raleigh, NC) as young adult males approximately seven weeks of age: mice (B6C3F1/CrlBR), Fischer rats (CDF(F-344)/CrlBR), Wistar rats (Crl:(WI)BR), and Golden Syrian hamsters (Lak:LVG(SYR)BR). Ranges of body weights at the time of gas uptake experimentation are given in Table 1. The animals were maintained in appropriate cages with rodent chow (Purina 5002) and water provided *ad libitum* and acclimated for at least 7 days prior to use. Laboratory facilities were fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). All procedures involving animals were reviewed by the laboratory animal care and use committee.

***Partition coefficients.*** Tissue-to-air partition coefficients (PCs) were quantified by modifying previously described procedures (Gargas *et al.*, 1989). Gas-tight vials (10 ml) in triplicate were prepared as reference vials or containing blood (1 g), lung (0.25 g), fat (0.15 g) or liver, muscle, or kidney (0.5 g). Tissues were applied directly to the vial as a smear on the vial walls, eliminating the need for a third saline-tissue reference vial (Gargas *et al.*, 1989). The vials were sealed and CD (100 ppm) was added after preheating to 37°C for 5 min in a vortex shaker (Labconco, Kansas City, MO). The vials were manually transferred to a programmable x-y-z robotic multi-purpose sampler (MPS2, Gerstel, Baltimore, MD) from which headspace samples (100 $\mu$l) were taken at 1.5, 3, and 4.5 h from the start of incubation. The gas chromatograph (GC) peak areas were obtained by electron capture detection using a HP6890 GC (Agilent Technologies, Wilmington, DE). Blank vials in triplicate were also analyzed. The partition coefficient (PC) was calculated using the following equation:

$$PC = [(GC_{area\ reference} * V_{vial} - GC_{area\ tissue} * (V_{vial} - V_{tissue})]/(GC_{area\ tissue} * V_{tissue}),$$

where V is the volume of the vial or tissue (Gargas *et al.*, 1989). Equilibration was generally established between 1.5 to 3.0 h. In some cases, equilibration was not observed in liver samples. Therefore, the time-zero intercept of the slope of the tissue-to-air concentration ratio curve for 1.5, 3.0, and 4.5 h samples was used to calculate the PC (Medinsky *et al.*, 1994). For measurement of the blood-to-air PC for humans, blood samples were drawn from three healthy male adult volunteers by a certified medical technologist and analyzed in triplicate.

***Closed-chamber exposures.*** Exposures were conducted using a system (Exposure System 1) described previously (Cantoreggi and Keller, 1997). The objective of the experiments with this system was to investigate chemical distribution with and without metabolic inhibition. Exposures were to initial concentrations ranging from 160 to 240 ppm CD. Some animals were pretreated with the cytochrome P450 monooxygenase inhibitor, 4-methyl pyrazole (4-MP, 144 mg/kg body weight; Chow *et al.*, 1992; Halpert *et al.*, 1994), prepared in saline and administered by ip injection 1 h prior to exposure. Animals ($n = 3$) were placed in the exposure chamber 30 min prior to the start of exposure. The chamber atmosphere was circulated through the system at approximately 2.0 l/min with a metal bellows pump (Metal Bellows, Sharon, MA). Tubing throughout the system was stainless steel and Teflon, and the total exposure system volume was 18 l for rats and 5.6 l for mice. Oxygen was monitored using an oxygen meter (MDA Scientific, Lincolnshire, IL) and carbon dioxide was scrubbed with soda lime. The concentration of CD in the chamber was monitored by GC/FID for up to 6 h. The inhalation system was connected to a gas chromatograph (Hewlett-Packard 5890A equipped with valve 18900 F). Samples of 250 $\mu$l were injected automatically every 10–12 min. The chromatography conditions were similar to those used for GC/FID analysis of CD *in vitro* metabolism (Himmelstein *et al.*, 2001a).

Additional gas uptake exposures in mice, Fischer rats, and hamsters were conducted using another system previously described (Evans *et al.*, 1994; McGee *et al.*, 1995). The purpose of this system (Exposure System 2) was to measure the uptake of CD over a range of initial starting concentrations. The system differed from Exposure System 1 in several ways: $O_2$ was added automatically instead of manually; $NH_3$ was scrubbed; one rat was used per chamber instead of three; and hamsters were substituted for Wistar rats. A known volume of concentrated CD vapor was added to the system having a total volume of 3.8 l. The starting exposure concentrations ranged from approximately 2 to 400 ppm for the mice and rats and 10 to 270 ppm for hamsters. The chambers were tested for leaks and considered operational when empty-chamber loss rates were $\leq$ 5%/h. Oxygen in the chamber was monitored continuously using a silver-electrode probe (Model 3300, MDA Scien-

TABLE 1
Physiological Parameters Used for Chloroprene PBTK Modeling

| Physiological parameters | Species | | | | |
|---|---|---|---|---|---|
| | Mouse | Fischer rat | Wistar rat | Hamster | Human |
| Values for dose-response modeling | | | | | |
| Body weight (kg)[a,b] | 0.03 | 0.25 | 0.25 | 0.11 | 70 |
| Ventilation (l/h/kg$^{0.75}$)[a,b] | 30 | 21 | 21 | 30 | 16.2 |
| Cardiac output (l/h/kg$^{0.75}$)[a,b] | 30 | 18 | 18 | 30 | 16.2 |
| Values for simulation of closed chamber gas uptake data | | | | | |
| Body weight (kg)[c] | 0.024–0.034 | 0.16–0.28 | 0.20–0.34 | 0.10–0.18 | na |
| Ventilation (l/h/kg$^{0.75}$)[c] | 15 | 10.5 | 10.5 | 12 | na |
| Cardiac output (l/h/kg$^{0.75}$)[c] | 15 | 9 | 9 | 12 | na |
| Tissue volumes as fraction of body weight[a,d] | | | | | |
| Liver | 0.055 | 0.04 | 0.04 | 0.04 | 0.026 |
| Fat | 0.05 | 0.07 | 0.07 | 0.07 | 0.214 |
| Rapid perfused | 0.035 | 0.05 | 0.05 | 0.05 | 0.077 |
| Slow perfused | 0.77 | 0.75 | 0.75 | 0.75 | 0.561 |
| Lung | 0.0073 | 0.005 | 0.005 | 0.005 | 0.0076 |
| Blood flow as fraction of cardiac output[a,d] | | | | | |
| Liver | 0.161 | 0.183 | 0.183 | 0.183 | 0.227 |
| Fat | 0.07 | 0.07 | 0.07 | 0.07 | 0.052 |
| Rapid perfused | 0.51 | 0.51 | 0.51 | 0.51 | 0.472 |
| Slow perfused | 0.15 | 0.15 | 0.15 | 0.15 | 0.249 |

*Note.* na, not applicable.

[a]Parameters for mouse, rats, and human based on Brown *et al.* (1997). Hamster ventilation, cardiac output, tissue volume, and tissue blood flow values were based on the mouse and rat.

[b]Values used for dose response modeling based on average body weight data from chronic inhalation studies and assumption that literature values based on Brown *et al.* (1997) for ventilation and cardiac output are representative of repeat inhalation exposure conditions.

[c]Values used specifically for simulation of closed chamber gas uptake data.

[d]Tissue volumes and blood flows are calculated by the model with resulting units of liters (l) and l/h, respectively.

tific, Lincolnshire, IL) and maintained between 19 and 21%. $CO_2$ and $NH_3$ were removed by circulating the system air through soda lime (Fluka) and sodium citrate (Aldrich), respectively. Animals were acclimated for 60 min prior to the introduction of test vapor. Chamber CD concentrations were monitored starting 10 min after vapor introduction and then every 10 min until the end of the exposure. An automatic gas sampling valve (250 $\mu$l) was used to introduce chamber air into the gas chromatograph for analysis by flame ionization or electron capture detection (Hewlett-Packard 5890A or Agilent 6890). The GC conditions were similar to those used for *in vitro* experiments (Himmelstein *et al.*, 2001a).

***Model development.*** A standard PBTK model was developed following the format used by Ramsey and Andersen (1984). The model consisted of distinct compartments for liver and lung, as well as lumped compartments for fat, slowly and rapidly perfused tissues (supplementary figure 1 is available at http://toxsci.oupjournals.org). Individual tissues were modeled as homogenous, well-mixed compartments connected by the systemic circulation. Modeled exposures occurred via partitioning into the arterial blood in the lung (Andersen *et al.*, 1987). Metabolism of CD was localized to the lung and the liver compartments and described by Michaelis-Menten type saturable enzyme kinetics. The model was used to estimate the CD concentration in each of the defined compartments as well as the blood. Model simulation was conducted using the Advanced Continuous Simulation Language (ACSL version 11.8.4, AEgis Technologies Group, Huntsville, AL). The model code is available upon request.

Standard physiologic values were used to parameterize the model (Table 1). Tissue-to-blood partition coefficients were calculated from the experimental tissue-to-air values. The *in vitro* metabolism parameters for total CD metabolism in liver and lung from Himmelstein *et al.* (2004) were scaled using microsomal protein content to yield *in vivo* parameters (Table 2). Microsomal protein content values for different species were derived from the literature. Reported liver protein concentrations were divided by a recovery factor of 0.32 reported for loss incurred because of the ultra-centrifugation preparation of the microsomes (Joly *et al.*, 1975). The values for liver were 35, 49, and 56.9 mg microsomal protein/g of tissue for mouse (Csanády *et al.*, 1992; Medinsky *et al.*, 1994), rats (Baarnhielm *et al.*, 1984; Boogaard *et al.*, 2000; Chiba *et al.*, 1990; Csanády *et al.*, 1992; Joly *et al.*, 1975), and humans (Lipscomb *et al.*, 2003a,b), respectively. The microsomal protein content for rats was used for the hamster. For lung microsomes, 23 mg microsomal protein/g tissue was used for all animal species assuming a protein recovery factor of 0.11 (Boogaard *et al.*, 2000; Medinsky *et al.*, 1994; Smith and Bend, 1980).

Gas uptake data were modeled by calculating the CD concentration in the chamber and subtracting the amount taken up by the animal. The physiological and metabolic parameters obtained from *in vitro* experimentation were not adjusted except for the alveolar ventilation (QPC) and cardiac output (QCC) as needed to adequately fit the experimental gas uptake data.

***Dose response analysis.*** The selection of the tissue dosimetry endpoints followed the approach recommended by Clewell *et al.* (2002). Because the known metabolism of CD involves two epoxide metabolites, but only one metabolite could be quantified *in vitro* (Himmelstein *et al.*, 2004), the dosimetric was based on the average daily total metabolism of CD divided by the tissue volume for liver or lung. The tissue dose definitions were milligram "amount-metabolized-parent-chemical" per gram of tissue per day in the liver (AMP) and lung (AMPLU). These were later calculated at the appropriate external rodent bioassay concentrations. PBTK model parameter sensitivity analysis for AMP and AMPLU were conducted using ACSL Math and Optimize version 2.5.4 (AEgis Technologies Group). Sensitivity coefficients, defined as the percent change in the prediction of interest as a result of a 1%

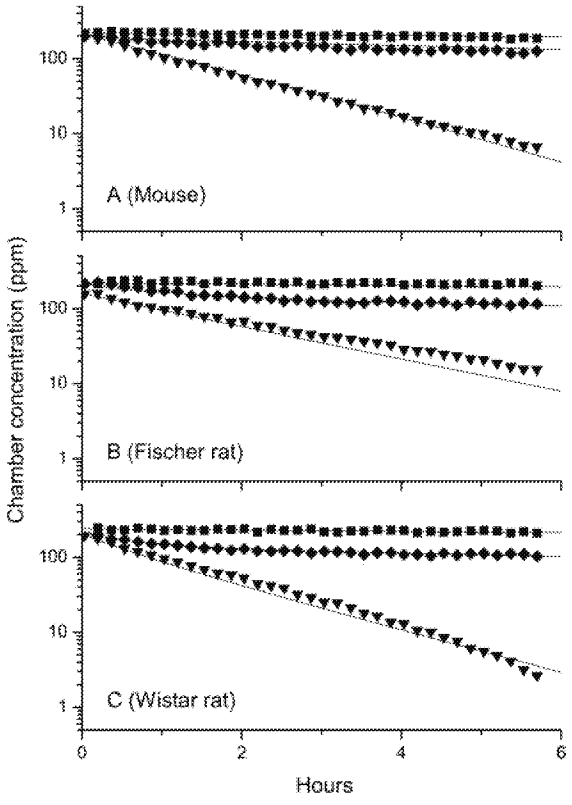


**FIG. 1.** *In vivo* closed chamber gas uptake of chloroprene: Experimental data (symbols) and model simulations (lines) representing control loss from deceased rats (■, solid square), rats pretreated with 4-methyl pyrazole (◆, solid diamond), or untreated (▼, inverted solid triangle). The target starting concentration was 200 ppm (actual range was 160 to 240 ppm for three animals per chamber and one chamber per exposure. Data were collected using Exposure System 1 described in the text.

increase in a given parameter value, were determined during steady-state exposure conditions and were normalized as recommended in Easterling *et al.* (2000) to correct for a known flaw in the computational algorithm. Only parameters with a sensitivity coefficient greater than 0.01 (1%) were considered to have a significant effect on the particular dosimetric.

AMPLU was selected for comparison with the incidence of combined bronchiolar adenoma/carcinoma in male rodents (Melnick *et al.*, 1999; Trochimowicz *et al.*, 1998). A dose response analysis for rodent liver was not possible because of a bacterial infection in the bioassay with male B6C3F1 mouse and the absence of a response in the male Fischer rat. The NTP (1998) concluded that the infection in male mice did not adversely affect the tumor responses in other tissues. The lung tumor incidence data for the B6C3F1 mouse and Fischer rat were the values corrected for intercurrent mortality using the 'poly-3' survival adjustment (Melnick *et al.*, 1999) or the total number of Wistar rats or Syrian hamsters examined (Trochimowicz *et al.*, 1998). The incidence data were corrected for extra risk equal to $(P_i - P_o)/(1 - P_o)$, where P is the probability of tumor incidence in "i" exposed and "o" control animals. Benchmark dose (BMD) modeling was performed using the multistage model of the USEPA Benchmark Dose Software (version 1.3.1) and a benchmark response of 10%. Initially, the analyses were performed with the definition of "dose" as exposure concentration (ppm) prior to adjustment for metabolism. Goodness of fit was based on visual inspection of the resulting graphical data and acceptance of a BMD *p*-value greater than 0.01.

The benchmark internal dose 95% lower bound (BMDL10%) was converted to a human equivalent exposure concentration (ppm) using the PBTK model. Human dosimetrics were also simulated over a wide range of CD exposure concentrations for continuous and discontinuous exposure scenarios. Human model exposures were run for up to two years. The 10% extra tumor risk corresponding to human exposure was determined by running the human PBTK model continuously (24 h/day, 365 days/year for two years) or discontinuously for two common workplace shift schedules (e.g., 8 h/day, 5 days/week or 12 h/day, 3 days/week for two years). Full lifetime exposure of 70 years (or 40 years for occupational exposure) was not necessary because the dosimetrics were calculated on a per day basis and reached a constant value after two simulation days.

## RESULTS

### *Model Development*

The three key steps of model development were the compilation of literature physiological parameters, calculation of metabolic parameters, and quantification of tissue-to-air PCs (Tables 1–3). The tissue-to-air PCs in Table 3 were entered into the PBTK model where they were transformed to tissue-to-blood PCs. Tissue-to-blood PCs were similar across animals and tissue types. The range of values were lung [1.0–2.9] ~ liver [1.1–2.3] ~ muscle [0.5 to 1.0] ~ kidney [0.9 to 2.6] < fat [14–28]. PCs were evaluated in the model sensitivity analysis described below, and predictions of AMP and AMPLU were sensitive to the blood-to-air PC and not PCs for the other tissues.

Data from the closed chamber gas uptake exposures were compared with model simulations of the declining chamber concentration. Inhibition of uptake was achieved with 4-MP pretreatment indicating that the decline of the CD chamber concentration was due to cytochrome P450 monooxygenase-mediated metabolism (Fig. 1). The difference between chamber control loss and metabolic inhibition represented uptake due to chemical distribution. A satisfactory model description for inhibition was obtained by setting the Vmax (Table 2) to zero for both liver and lung metabolism (Fig. 1). Exposures over a wider range of starting exposure concentrations using

**TABLE 2**
**Metabolic Parameters**

| Biochemical parameters[a] | Species: Mouse | Fischer rat | Wistar rat | Hamster | Human | Units |
|---|---|---|---|---|---|---|
| Liver metabolism | | | | | | |
| Vmax | 39.2 | 11.50 | 15.5 | 42.8 | 9.1 | mg/h/kg bw |
| Km | 0.091 | 0.047 | 0.075 | 0.118 | 0.060 | mg/l |
| Vmax/Km | 431 | 244 | 208 | 363 | 152 | l/h/kg bw |
| Lung metabolism | | | | | | |
| Vmax | 1.02 | — | — | — | — | mg/h/kg bw |
| Km | 0.13 | — | — | — | — | mg/l |
| Vmax/Km | 7.67 | 0.14 | 0.14 | 0.14 | 0.14 | l/h/kg bw |

[a]Scaled from Himmelstein *et al.* (2004) using microsomal protein contents described in the text.

TABLE 3
Tissue-to-Air Partition Coefficients

| Tissue | Tissue-to-air partition coefficients (mean ± SE)[a] | | | | |
|---|---|---|---|---|---|
| | Mouse | Fischer rat | Wistar rat | Hamster | Human |
| Blood | 7.8 ± 0.1 | 7.3 ± 0.1 | 8.0 ± 0.5 | 9.3 ± 0.3 | 4.5 ± 0.1[b] |
| Lung | 18.6 ± 5.1 | 13.5 ± 1.6 | 11.2 ± 0.5 | 9.7 ± 0.6 | 13.3 ± 4.1[c] |
| Liver | 9.8 ± 0.9 | 11.5 ± 0.3 | 10.9 ± 0.2 | 10.5 ± 0.5 | 10.7 ± 1.1[c] |
| Fat | 135.3 ± 1.6 | 124.0 ± 1.5 | 126.3 ± 1.4 | 130.1 ± 0.9 | 128.9 ± 2.7[c] |
| Muscle[d] | 4.6 ± 0.8 | 4.4 ± 0.4 | 4.0 ± 0.3 | 5.0 ± 0.2 | 4.5 ± 1.0[c] |
| Kidney[e] | 13.7 ± 0.6 | 16.7 ± 0.6 | 9.4 ± 0.4 | 8.2 ± 0.3 | 12.0 ± 0.9[c] |

[a]Mean ± standard error (SE) for three replicates per rodent tissue unless noted otherwise.
[b]Human CD blood value for nine replicates (three subjects, three vials/subject).
[c]Means and SE derived from rodents; the SE was adjusted to account for the proportion of variation from each set of rodent data.
[d]Used to represent the slowly perfused tissue group.
[e]Used for the rapidly perfused tissue group.

the B6C3F1 mouse, Fischer rat, and hamster also demonstrated good agreement with model simulations (Fig. 2). The range of starting concentrations were selected to span above and below the bioassay concentrations.

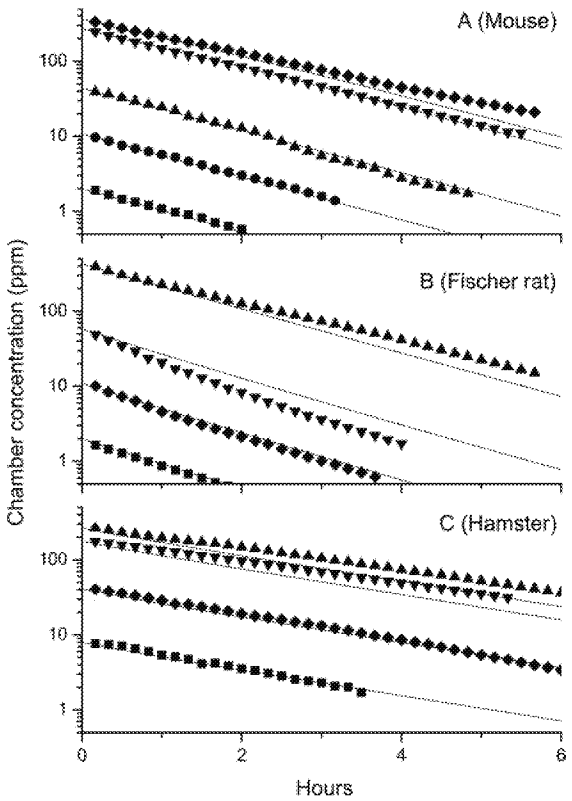


FIG. 2. *In vivo* closed chamber gas uptake of chloroprene: Mean experimental data (symbols) and model simulations (lines) for (A) B6C3F1 mouse at initial concentrations of 2, 10, 50, 270, or 363 ppm (three animals per chamber, two chambers/exposure), (B) Fischer rat at 2, 11, 58, or 433 ppm (one animal per chamber, two chambers/exposure), and (C) hamster at 8, 41, 178, or 267 ppm (one animal per chamber, four chambers/exposure). Data were collected using Exposure System 2 described in the text.

For both exposure systems, *in vitro* scaling of total CD metabolism was sufficient to explain the *in vivo* gas uptake data. The alveolar ventilation and cardiac output values used for simulation of the experimental gas uptake data were lower than the standard values used for dosimetry modeling (Table 1). The adjustment for the gas uptake simulations gave values for alveolar ventilation that were consistent with those used for modeling of various chemicals (Johanson and Filser, 1992; Medinsky *et al.*, 1994). Plausible explanations proposed by Johanson and Filser (1992) for using approximately 60% of the theoretical alveolar ventilation values reported by Arms and Travis (1988) included reduced ventilation due to sensory irritation, absorption and desorption by the upper airways, or anesthetic effects. For dosimetry modeling, the decision was made to assume the standard ventilation and cardiac parameters based on Brown *et al.* (1997) given the possibility that these parameters were more appropriate for estimating uptake and metabolism associated with bioassay conditions involving repeated whole body exposure.

### *Internal Dose*

The known lung tumor incidence in male B6C3F1 mouse and the male Fischer rat, and the major role that the liver has in the overall metabolism of CD drove the selection of these tissues for internal dose estimation. A key finding was that the internal dose for total CD metabolism per gram lung per day was greater in the mouse than in the rats or hamster (Table 4). The liver internal dose (AMP) was linear over the range of the bioassay concentrations (data not shown). The lung internal dose (AMPLU) was linear for the rats and hamster but suggests saturable behavior for the mouse (Table 4). As noted above, a *Helicobacter* infection in the male B6C3F1 mouse bioassay excluded the use of the liver tumor incidence for dose response analysis (Melnick *et al.*, 1999). The NTP (1998) assumed that the infection did not alter the

**TABLE 4**
**Exposure-Dose-Response for Rodent Lung Tumors**

| Species | Exposure concentration (ppm) | PBTK internal dose[a] | Lung tumor incidence | Number of animals | Extra risk incidence (%)[b] |
|---|---|---|---|---|---|
| Hamster[c] | | | | | |
| | 0 | 0 | 0 | 100 | 0 |
| | 10 | 0.18 | 0 | 97 | 0 |
| | 50 | 0.88 | 0 | 97 | 0 |
| Wistar rat[c] | | | | | |
| | 0 | 0 | 0 | 97 | 0 |
| | 10 | 0.18 | 0 | 13 | 0 |
| | 50 | 0.89 | 0 | 100 | 0 |
| Fischer rat[d] | | | | | |
| | 0 | 0 | 3 | 50 | 0 |
| | 12.8 | 0.22 | 3 | 50 | 0.3 |
| | 32 | 0.55 | 6 | 49 | 7.7 |
| | 80 | 1.37 | 9 | 50 | 14.0 |
| B6C3F1 mouse[d] | | | | | |
| | 0 | 0 | 15 | 50 | 0 |
| | 12.8 | 3.46 | 32 | 50 | 48.3 |
| | 32 | 5.30 | 40 | 50 | 70.4 |
| | 80 | 7.18 | 46 | 50 | 89.9 |

[a]Internal dose = average daily mg CD metabolized/g lung tissue (AMPLU).
[b]See text for explanation of extra risk calculation.
[c]Male Hamster and Wistar rat data from Trochimowicz *et al.* (1998).
[d]Male Fischer rat and B6C3F1 mouse data from Melnick *et al.* (1999).

response of other tumor types, thus metabolism was assumed to be unaltered as well.

### *Sensitivity Analysis*

Sensitivity analysis showed that parameters such as the blood-to-air partition coefficient, ventilation rate, cardiac output, liver blood flow, and body weight had sensitivity coefficients greater than 0.2 (20%), and thus were most important for estimating how much daily average metabolism of parent chemical occurred in each tissue (Fig. 3). The kinetic parameters for lung metabolism also were found to be significant for the mouse; but this was not the case for the first-order kinetic parameters of lung metabolism used for the other species (Table 2), despite the fact that the lung receives 100% of the total blood flow. The analysis also indicated that CD metabolism in the liver was limited by mass transport (blood flow) rather than metabolism.

### *Model Application*

The procedure for model extrapolation from rodents to humans was consistent with the description provided by Clewell *et al.* (2002). The concepts of dosimetry modeling described by Clewell *et al.* (2002) also supported the use of total metabolism in the current context independent of animal species and metabolic product. A key assumption was that total CD metabolism leads to reactive metabolites. Combining the tumor response data across species for dosimetry modeling was similar to the approach used by Kirman *et al.* (2000) to evaluate how differences in sensitivity may be related to metabolism and to aid cross species extrapolation. Applicability of the PBTK model was clearly demonstrated by relating lung metabolism to lung tumor response. No interspecies dose response was evident using exposure concentration (ppm) as the applied dose (Fig. 4). When internal dose AMPLU was used, the greater metabolism in the male mouse lung correlated with the greater magnitude of response relative to the rats and hamster. The 95% lower bound internal dose corresponding to a 10% extra tumor risk (BMDL10%) from the combined B6C3F1 mouse, Fischer and Wistar rat, and hamster incidence data was 1.3 mg CD metabolized per gram tissue per day. Human simulations were performed from 0 to 1250 ppm to identify exposure concentrations equivalent to the BMDL10% internal dose. The

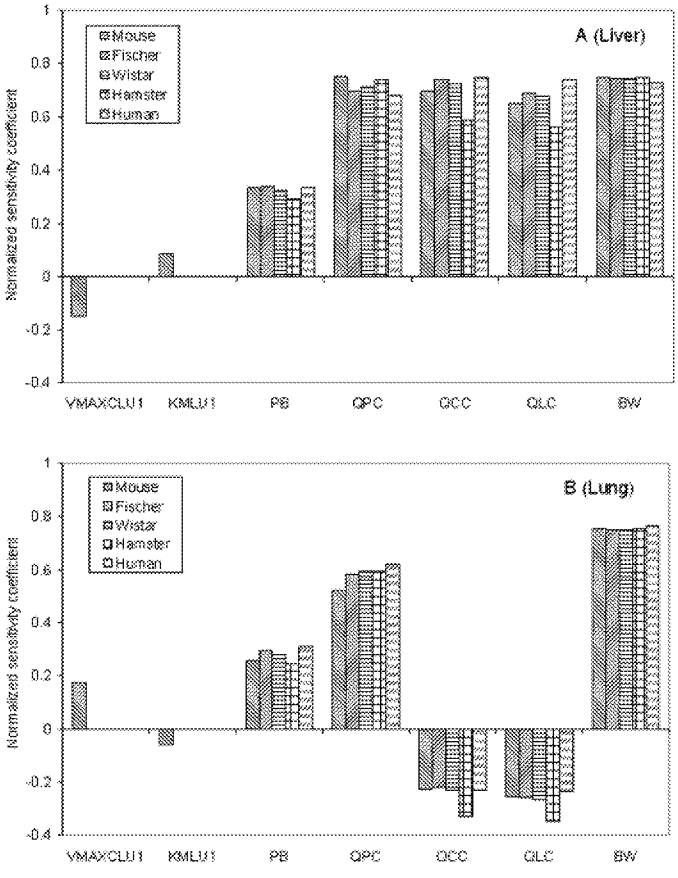


**FIG. 3.** Sensitivity analysis showing normalized coefficients for total chloroprene metabolism by the liver (A) or lung (B), obtained during steady state exposure to chloroprene. Similar coefficients were obtained at 12.8, 32, or 80 ppm chloroprene exposure; data are shown for the 32 ppm exposure. Only normalized coefficients showing a change greater than a 0.01 (1%) in the respective dosimetric prediction are presented. These include Vmax and Km for lung metabolism (VMAXCLU1, KMLU1), the blood-to-air partition coefficient (PB), alveolar ventilation (QPC), cardiac output (QCC), liver blood flow (QLC), and bodyweight (BW). Units are given in the respective parameter tables.

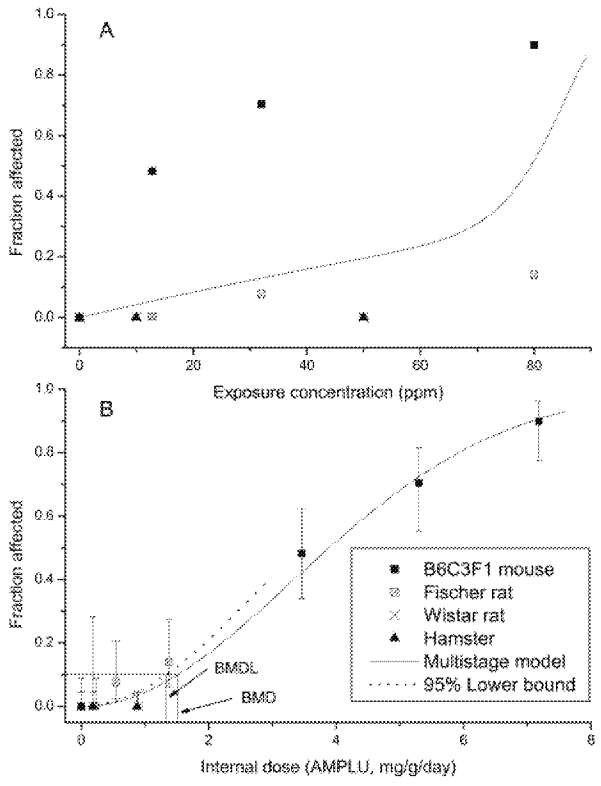


FIG. 4. Multistage benchmark dose (BMD10%) model with 95% confidence level (BMDL10%) for fraction of animals affected by lung tumors. Fraction affected as extra risk compared with external exposure concentration (panel A) or internal dose of chloroprene metabolism (panel B) in the lung. Panel B includes curves for BMD and BMD 95% lower bound and model derived confidence intervals for the respective extra risk values. The BMD model $p$-value was 0.0107; only the BMD curve is given in panel A due to poor fit (BMD $p$-value = 0.0000). The fraction affected which was corrected for extra risk and the derivation of internal dose is described in the text.

human model simulations of total CD metabolism in lung or liver were normalized on a per day basis and reached a constant value after two simulation days, indicating that the length of the simulation was not a factor in the predicted internal dose. Values for AMPLU were the same after two days or two years. The human AMPLU was linear to 500 ppm CD (data not shown). Three relationships for the human internal dose AMPLU and the human equivalent concentration up to 200 ppm are shown in Figure 5; one for continuous exposure (24 h/day), and two for discontinuous exposure as 8 h/day or 12 h/day shift work. External exposure concentrations, corresponding to the same BMDL10% were 4.3 to 4.7 times greater for the discontinuous exposures compared with continuous exposure. The resulting estimates of external exposure concentrations for continuous and discontinuous exposure, which are equivalent to time weighted average concentrations, can be used as points of departure for subsequent risk assessment.

## DISCUSSION

The results of this study demonstrate an interspecies estimate of lung tumor risk through the combination of PBTK modeling and BMD analysis. The PBTK model development was undertaken with the knowledge that exposure concentration was not the best dosimeter (Fig. 4A). For example, the extra risk incidence of male mouse lung tumors was greater than 48% at the lowest bioassay concentration of 12.8 ppm. The shape of the dose response curve at lower exposure concentrations could not be described reliably given this high incidence (EPA, 2000). Accounting for the species differences in lung metabolism among the rodents provided a more complete view of the low dose-response region and the ability to extrapolate the dose response curve to humans using *in vitro* metabolism data specific for each species. The current PBTK model specifically accounted for CD uptake due to distribution and metabolism. In this manner the current model captured the flux of CD to its metabolites. *In vitro* metabolism studies in this laboratory and by others (Cottrell *et al.*, 2001; Himmelstein *et al.*, 2001a) identified two epoxide metabolites, but the quantitative kinetic rates describing the balance of formation and elimination are still uncertain (Himmelstein *et al.*, 2004). Hence the development of PBTK submodels and the potential direct use of epoxide dosimetry would require further extensive experimentation.

The selection of PBTK model parameters was dependent on the suspected mode of action in the animal species used in the bioassay studies. To the extent possible, metabolic and partitioning parameters were quantified in human tissues using a parallelogram approach that has been applied to other chemicals (e.g., Csanády *et al.*, 1992; Medinsky *et al.*, 1994). Results for the tissue-to-air partition coefficients were consistent with

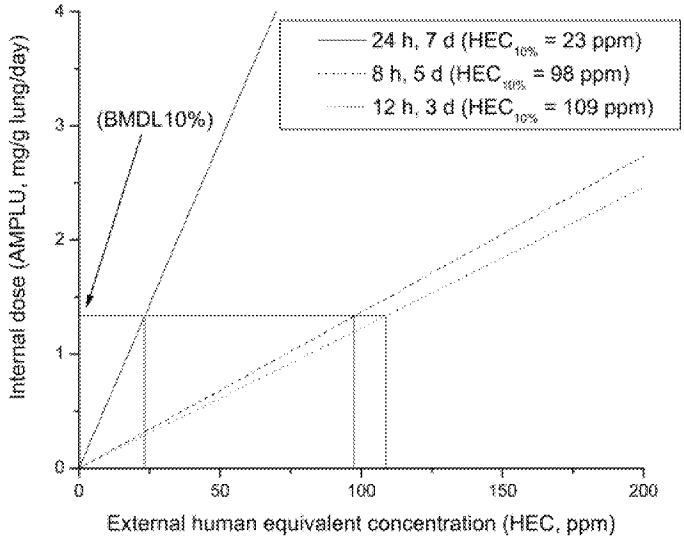


FIG. 5. Chloroprene inhalation human equivalent concentration for 10% extra risk ($HEC_{10\%}$). The HEC relates to the rodent derived internal dose (BMDL10%) generated from the PBTK model.

values reported by Gargas *et al.* (1989) for a variety of volatile organic chemicals, who generally observed 1.5 to 2 fold higher blood-to-air partition coefficients in rat blood compared with human blood. These parameters, and the physiological parameters adopted directly from Brown *et al.* (1997) carried the least inherent uncertainty. One exception was the reduced alveolar ventilation and total blood flow values required for simulation of the closed-chamber gas uptake concentration data. This modification was consistent with values for alveolar ventilation used in the gas uptake literature for various volatile chemicals (Johanson and Filser, 1992; Medinsky *et al.*, 1994). The lowering of alveolar ventilation paired with cardiac output reduced the uptake of CD and gave the best overall description of the gas uptake data. Changing the scaled metabolic parameters to increase or decrease uptake was not performed because the kinetic parameters were determined experimentally. The gas uptake experiments in the current project served to verify the scaled *in vitro* metabolism parameters. The *in vivo* Vmax values for CD compared reasonably well with those for vinyl chloride and 1,3-butadiene (1.1 and 3.2–12.8, respectively, versus 9.1 mg/h/kg body weight for CD). The Km values were also generally consistent with values of 0.04, 0.11–0.28, and 0.06 mg/l (Medinsky *et al.*, 1994; Reitz *et al.*, 1996; Sweeney *et al.*, 2001). The liver was the major contributor to total body metabolism based on comparison of Vmax/Km with liver blood flow. For example, the Vmax/Km (152 l/h/kg) was considerably greater than liver flow calculated from Table 1 (1.8 l/h/kg = $16.2^{0.75}$ * 0.227) for the human. Thus, the overall metabolism is most likely limited by blood flow perfusion as reported for other cytochrome P450 monooxygenase volatile substrates (Lipscomb *et al.*, 2003b; Sweeney *et al.*, 2001).

Scaling *in vitro* metabolism from liver and lung microsomal fractions is a generally accepted practice, but introduces model uncertainty. The uncertainty comes from the correction factor for loss of protein during centrifugal preparation of the microsomal fractions. Reasonable estimates were available for liver and lung from the literature (see Materials and Methods). Recent experiments describing the recovery of human liver microsomal protein provide additional confidence for protein scaling (Lipscomb *et al.*, 2003a). The same investigators have also concluded that unsaturated metabolism of trichloroethylene by cytochrome P450 monooxygenase is flow limited, more than likely minimizing any major impact of microsomal protein content on metabolic variation (Lipscomb *et al.*, 2003b). Sensitivity analysis for the current study adds an additional perspective that physiological parameters were more important than metabolic parameters for prediction of internal tissue dose AMP and AMPLU associated with cytochrome P450 monooxygenase mediated metabolism of CD (Fig. 3). Using pooled human liver and lung microsomal fractions is also a source of uncertainty in that this approach does not account for the impact that human interindividual variation might have on metabolism. Further considerations of model uncertainty beyond this current effort may be needed if data from pooled microsomes are considered insufficient for application to risk assessment.

The use of PBTK-derived CD tissue dosimetry for exposure-dose-response modeling for this project was similar to previous descriptions of dichloromethane and vinyl chloride (Andersen *et al.*, 1987; Reitz *et al.*, 1989, 1996). In both cases, PBTK models were used to derive life-time average daily production of metabolite divided by liver tissue volume. The significant finding from the current project was that BMD modeling based on CD metabolism in lung tissue (AMPLU) described the greater mouse lung tumor incidence compared with other rodents. Overall metabolism by lung was considerably lower than metabolism by the liver. The exposure-dose-response modeling was applied to the predictions of AMPLU and corresponding external human equivalent exposure concentrations for CD from continuous and discontinuous exposure scenarios (Fig. 5).

Despite the less than complete understanding of mechanisms leading to tumor development, the mode of action for CD-induced tumorigenicity most likely involves metabolic activation and genotoxicity due to interaction with DNA and other cellular components (see Introduction for literature cited). The mode of action appears to be similar to chemical analogs 1,3-butadiene and isoprene (Himmelstein *et al.*, 1997; Watson, 2001). For example, 1,3-butadiene exposure caused lung tumors in the B6C3F1 mouse. For CD, the plausible mode of action and the greater mouse lung tumor response relative to the other rodents indicate the acceptability of the available data for risk extrapolation. The approach used generally adhered to the principles described in the EPA carcinogen risk assessment guidelines (EPA, 2003). One key step was pooling of the rodent lung tumor response data. Successful cross-species pooling of response data using PBTK modeling was recently demonstrated for acrylonitrile-induced brain tumors in rats (Kirman *et al.*, 2000). The internal dose measure (AMPLU) for CD greatly improves the understanding of the lower lung tumor response for the Fischer rat and lack of response for the Wistar rat and Syrian hamster compared with the B6C3F1 mouse. Strain differences in detoxification of the reactive metabolites, which the current model does not address, may explain the greater sensitivity of the Fischer rat compared with the Wistar rat. Nonetheless, the results provide a much better description of interspecies dose response in the 10% percent extra tumor risk range that is considered representative of the range of sensitivity for bioassay studies.

In conclusion, development of a human PBTK model, in conjunction with corresponding physiological, partitioning, and metabolic parameters in rodents, led to a useful demonstration of exposure-dose-response modeling. PBTK simulation of continuous exposure in humans predicted an external concentration of 23 ppm CD to match the corresponding internal dose BMDL10% from all the rodent lung tumor data. Discontinuous workplace exposure resulted in approximately 4 to 5 fold higher external exposure concentrations. These hu-

man equivalent concentrations could serve as points of departure for extrapolation to an acceptable risk range.

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